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Increased Endogenous Abscisic Acid Maintains Primary Root Growth and Inhibits Shoot Growth of Maize Seedlings at Low Water Potentials[1]

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ABSTRACT

Roots of maize (*Zea mays* L.) seedlings continue to grow at low water potentials that cause complete inhibition of shoot growth. In this study, we have investigated the role of abscisic acid (ABA) in this differential growth sensitivity by manipulating endogenous ABA levels as an alternative to external applications of the hormone. An inhibitor of carotenoid biosynthesis (fluridone) and a mutant deficient in carotenoid biosynthesis (vp 5) were used to reduce the endogenous ABA content in the growing zones of the primary root and shoot at low water potentials. Experiments were performed on 30 to 60 hour old seedlings that were transplanted into vermiculite which had been preadjusted to water potentials of approximately −1.6 megapascals (roots) or −0.3 megapascals (shoots). Growth occurred in the dark at near-saturation humidity. Results of experiments using the inhibitor and mutant approaches were very similar. Reduced ABA content by either method was associated with inhibition of root elongation and promotion of shoot elongation at low water potentials, compared to untreated and wild-type seedlings at the same water potential. Elongation rates and ABA contents at high water potential were little affected. The inhibition of shoot elongation at low water potential was completely prevented in fluridone-treated seedlings during the first five hours after transplanting. The results indicate that ABA accumulation plays direct roles in both the maintenance of primary root elongation and the inhibition of shoot elongation at low water potentials.

A major reason behind the slow progress in the area of crop adaptation to drought is the insufficient basic understanding of the regulation of growth responses to water stress. When water is limited, shoot growth in many species is more inhibited than root growth (24), and in some cases, the absolute biomass of roots has been shown to increase relative to well-watered controls (12, 23). In maize, roots continue to grow at low water potentials that cause complete inhibition of shoot growth (25, 30). The role of the hormone ABA in the differential growth responses of the primary root and shoot of maize to low water potentials is the subject of this paper.

ABA accumulates to high concentrations in tissues of plants experiencing water stress and has been proposed to be involved in the regulation of root and shoot growth responses (3, 5, 6). Speculations on the involvement of ABA in growth responses to water stress have relied on the results of ABA applications to well-watered plants. Such applications have generally led to inhibition of shoot growth (1, 5, 11, 28, 29). In the case of roots, results have been variable; applications of ABA have resulted in growth inhibition (10, 18, 29), promotion (15, 19, 29), or have had little effect (5). In maize, applications of the hormone led to stimulation or inhibition of primary root growth depending on the initial root growth rate (19) and the concentration used (15, 19), with higher concentrations being associated with growth inhibition. Additionally, in a population of maize primary roots with varying growth rates, higher levels of endogenous ABA were associated with slower growth rates (22). Nevertheless, the lack of inhibition and, in some cases, stimulation of root growth by some concentrations of exogenous ABA has led to speculation that ABA may modulate the differential inhibition of root and shoot growth in water-stressed plants (5, 9, 29, 31).

When ABA is applied to well-watered plants, however, even if it accumulates to realistic levels in the growing zone (5), its compartmentation may differ from that of endogenous ABA in water-stressed tissue (8). Also, other metabolic changes that may be induced by water stress, such as changes in the levels of other hormones and tissue sensitivity to hormones, are not addressed when well-watered tissue is used to study the involvement of ABA in growth responses to low water potentials. Thus, it is not surprising that in one example ABA applications produced different growth responses in well-watered and water-stressed plants (29). Consequently, the role, if any, of ABA in the growth responses of roots and shoots to low water potentials remains equivocal.

With this in mind, our aim was to critically evaluate the role of elevated levels of endogenous ABA in the growth responses of the primary root and shoot of maize to low water potentials, without exogenous applications of the hormone. Specifically, our objective was to reduce the accumulation of endogenous ABA in seedlings growing at low water potential using two approaches: a mutant deficient in carotenoid (and ABA) biosynthesis (vp 5, [7, 14]), and fluridone, an inhibitor of carotenoid (and ABA) biosynthesis (7, 13) applied to seedlings of a commercial variety. Use of the two approaches was intended to circumvent any side effects that the inhibitor may have on growth independently of ABA content, and to confirm results obtained using one approach. The results indicate

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that the accumulation of endogenous ABA at low water potentials acts differentially to maintain primary root growth and inhibit shoot growth.

MATERIALS AND METHODS

Primary Root Growth

For experiments with fluridone, seedlings of *Zea mays* L. (cv FR27 × FRMo17) were germinated in moist vermiculite and transplanted after 36 h into vermiculute of either high or low water content, corresponding to water potentials of approximately −0.03 MPa and −1.6 MPa, as described previously (25). The same lot of vermiculite, thoroughly mixed, was used for all the experiments to obtain approximately the same water potential at the same water content. Vermiculite water potential was measured by isopiestic thermocouple psychrometry (2). Seedlings were grown in Plexiglas boxes in the dark at 29 ± 1°C and near-saturation humidity. Preliminary tests determined that fluridone at 10 μM had little effect on root growth rate at high water potential. Therefore, for the fluridone treatments, fluridone was added at this concentration to the water mixed with the vermiculite in which the seeds were germinated and into which the seedlings were transplanted. Fluridone was supplied by Lilly Research Laboratories. Total root length was determined either by marking the position of the root apices on the angled (7° from vertical) Plexiglas face against which the roots were growing (under green light; maximum transmission: 512 nm, range: 460–560 nm), or, in the fluridone treatment at low water potential, by destructive harvesting at various times. This was necessary because, in this case, many roots grew into the vermiculite and were thus not visible.

Vp 5 kernels were supplied by Dr. E. Coe (Department of Agronomy, University of Missouri-Columbia). Mutant and wild-type kernels were produced by self-pollination from heterozygous parent seed provided by Dr. J. D. Smith (Department of Soil and Crop Sciences, Texas A&M University). The background of the cross was at the second-backcross level with inbred line TX5855. As mutant kernels are viviparous, stocks are usually maintained in heterozygous condition, deriving homozygotes by selfing. Despite the viviparous tendency, however, some mutant kernels do not undergo visible precocious germination, possibly because of incomplete blockage of ABA biosynthesis or because ABA is supplied from maternal cob tissue. Thus, these mutant kernels mature in a similar fashion to wild-type kernels. Because wild-type kernels must undergo desiccation before they will germinate, it was crucial that mutant kernels undergo the same treatment to eliminate desiccation as a variable which might affect seedling growth at low water potential. Mutant kernels which survived desiccation on the plant were tested for germination and only those which showed a similar root growth rate to wild-type kernels were used in the experiments. As a result of these experimental conditions, however, the number of usable mutant seed was limited. Mutant and wild-type seedlings were grown and root length was measured as described above except that no fluridone was added to the vermiculite.

Shoot Growth

The growth response of the maize shoot to low water potential was also studied using both the inhibitor and mutant approaches to manipulate endogenous ABA levels. Because shoot growth is more inhibited than root growth at low water potentials (25), we selected a low water potential treatment (approximately −0.3 MPa) that resulted in a similar percentage inhibition of shoot elongation rate to that which occurred in the root at −1.6 MPa (approximately 60% inhibition). For these experiments, seedlings were transplanted to the high and low water potential treatments at 60 h after planting since tests showed that shoot development is greatly retarded if low water potential is imposed after only 36 h. Seedlings were grown in Plexiglas cylinders (0.5 m × 0.1 m), and growing conditions and shoot length measurements (nondestructive) were as described for studies of the primary root.

Time Course of ABA Content

At various times after transplanting, the apical 10 mm of the primary roots (encompassing the growing zone [25]) were harvested under near-saturation humidity and dim white or green light, weighed before and after freeze drying, and assayed for ABA. ABA levels were quantified only two times during the experiments with the mutant and wild type due to the limited availability of seed. Each sample consisted of 3 to 6 root tips. Most of the root cap was excluded from the samples by excising the apical 0.5 mm.

For the shoots, the growing zones were localized by a marking procedure as previously described for the primary root (25), and results indicated that elongation occurred in the coleoptile and the uppermost 12 to 15 mm of the mesocotyl. Local growth rates in the upper mesocotyl were higher than in the coleoptile, however, so ABA quantification was made in the mesocotyl region only. At various times after transplanting, the uppermost 15 mm of the mesocotyls were harvested under near-saturation humidity and green light, weighed before and after freeze drying, and assayed for ABA. ABA levels were quantified only two times during the experiments with the mutant and wild type due to the limited availability of seed. Each sample consisted of 2 to 5 mesocotyl sections.

In addition to selecting for uniformity of development at transplanting, roots and shoots used for ABA quantification were visually selected at harvest time for having growth rates near the mean growth rate for the respective treatments. This was important in light of the finding that ABA content can be highly variable in a population of roots with a wide range of growth rates (22).

ABA Quantification

ABA levels were quantified in extracts of root and shoot growing zones by a radioimmunoassay (RIA) obtained from Dr. S.A. Quarrie (AFRC Institute of Plant Science Research, Cambridge, England). Tissues were extracted in cold (4°C) water for 16 to 20 h because tests in our laboratory and elsewhere (20) determined aqueous extraction to be as efficient as extraction with organic solvents. Assay procedures

were as described by Quarrie *et al.* (20). We observed that sequential addition of standard ABA solutions to extracts of root and shoot growing zones at high and low water potentials produced 1:1 relationships between ABA added and total ABA measured by RIA, indicating the absence of interference with binding of the antibody to ABA. In addition, because no reports have been published on the use of this assay on extracts of maize root and shoot growing zones, RIA results were compared with results obtained by combined gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring as described below. ABA values obtained by RIA were near identical to those obtained by GC-MS for root and shoot tissues at low water potential. For root and shoot tissues at high water potential, ABA values obtained by RIA were 50% to 75% higher than those obtained by GC-MS, indicating some cross-reactivity with other compounds. The over-estimation of the reported ABA contents at high water potential, however, does not affect our interpretation of the results.

For GC-MS validation, ($^2H_6$) (±)-ABA was prepared as described by Rosher *et al.* (21), and was added prior to extraction as an internal standard. The procedure for extraction and initial purification was as described by Stewart *et al.* (27). Partially purified samples were further purified using a dual pump HPLC system (ISCO). Aliquots were injected onto a C-18 column (Spherisorb 5 μm ODS, 250 × 10 mm i.d., from Phenomex) which had been equilibrated with 10:90 acetonitrile:aqueous 200 mM acetic acid. The samples were eluted with a gradient of 25 to 50% acetonitrile in aqueous 200 mM acetic acid over a period of 18 min at 2.5 mL min^{-1}, followed by 10 min of 100% acetonitrile. Fractions eluting at the retention time of ABA were collected, evaporated to dryness and redissolved in isopropanol. An aliquot of the isopropanol solution was injected onto an amino column (5 μm NH_2, 250 × 4.6 mm i.d., from ISCO) and eluted isocratically with 80:20 hexane:200 mM acetic acid in isopropanol at 1.5 mL min^{-1}. The ABA fractions were again collected and dried, samples were derivatized with diazomethane in ether, and were then evaporated to dryness. The residue was dissolved in methylene chloride, and aliquots were injected onto a 25 m, 0.2 mm i.d. cross-linked methyl silicone column (Hewlett Packard) in a Fractovap GC (Carlo Erba, Milan, Italy). After a 3-min delay at 80°C, the column was heated at 40°C min^{-1} to 280°C, then held for 5 min at 280°C. The injector temperature was 280°C. Helium was the carrier gas, with an inlet pressure of 16 psi. A Kratos MS25 (Kratos Analytical, Inc.) was used for mass spectroscopy. Ions at m/z of 190 and 194 were monitored. GC-MS procedures were performed at the University of Missouri Center for Mass Spectrometry.

Data Presentation

Experiments were repeated as indicated in the figure legends. Representative experiments are shown for clarity except for treatments that required destructive harvesting, in which case combined experiments are shown. Root growth rates in the text were calculated from linear regression lines fitted to the data in the figures. For the shoots, average growth rates over the first 5 to 6 h after transplanting are reported. ABA levels are expressed as ng ABA g H_2O^{-1} to reflect the volume-averaged concentration in the growing zone. This, however, does not necessarily reflect the physiological concentration at the active site(s) since the tissues were homogenized and extracted as a whole.

RESULTS

Effect of Fluridone on Root Growth at High and Low Water Potential

Treatment with fluridone was associated with reduced primary root growth at low water potential (Fig. 1). Root elongation rate of fluridone-treated seedlings was reduced by 83% during the first 30 h after transplanting to low water potential (approximately −1.6 MPa) vermiculite, compared with untreated roots at high water potential (0.4 mm h^{-1}compared with 2.4 mm h^{-1}). Root elongation rate of untreated seedlings was reduced by only 55% at the same water potential (1.1 mm h^{-1}). Treatment with fluridone had little effect on root elongation rate at high water potential (6–16% reduction in three replicate experiments).

At approximately 50 h after transplanting to the low water potential vermiculite, root elongation rate of fluridone-treated seedlings began to increase, and after approximately 120 h

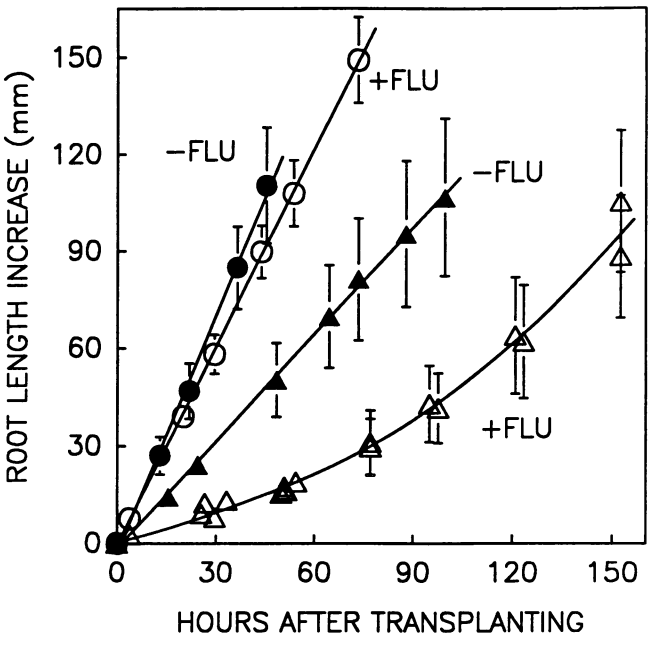

Figure 1. Effect of fluridone (FLU) on elongation of the primary root of maize (FR27 × FRMo17) seedlings growing in high (−0.03 MPa, circles) or low (−1.60 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 20 to 40 roots. For the +FLU low water potential treatment, data points are combined from five experiments needed to cover the time course because of destructive harvesting for length measurement. For other treatments, data points are from single experiments which were repeated 1 to 6 times with similar results. Error bars are not shown when smaller than the symbols.

had recovered to that of untreated seedlings at the same water potential. This response was observed in repeated experiments (Fig. 1). A likely explanation is that water uptake by roots at −1.6 MPa may have occurred mostly from the vapor phase, resulting in progressive dilution of the fluridone present in the tissue prior to transplanting and a concomitant increase in growth rate.

Root Growth of Mutant and Wild Type at High and Low Water Potential

Results of experiments with the vp 5 mutant and wild type were similar, respectively, to those obtained with fluridone-treated and untreated seedlings of the commercial variety (*cf.* Figs. 1 and 2). At −1.6 MPa, root elongation rate of the mutant was reduced by 86% compared with the wild type at high water potential (0.3 mm h^{-1} compared to 2.1 mm h^{-1}; Fig. 2). Root elongation rate of the wild type was reduced by only 52% at the same water potential (1.1 mm h^{-1}). At high water potential, root elongation rate of the mutant was 16% less than that of the wild type (observed in two replicate experiments).

Time Course of ABA Content in the Root Growing Zone

The time course of ABA content was measured in the apical 10 mm of the roots to document that accumulation of ABA was indeed reduced in the growing zone of fluridone-treated and mutant seedlings at low water potential (Fig. 3; Table I). As expected, low water potential induced a large increase in the ABA content in untreated and wild-type seedlings. In fluridone-treated and mutant seedlings at the same water potential, the accumulation of ABA was reduced considerably. At approximately 120 h after transplanting, ABA content in fluridone-treated roots at low water potential recovered to that found in untreated roots at the same water potential (Fig. 3). There was little effect of either fluridone treatment or the mutant on ABA content at high water potential.

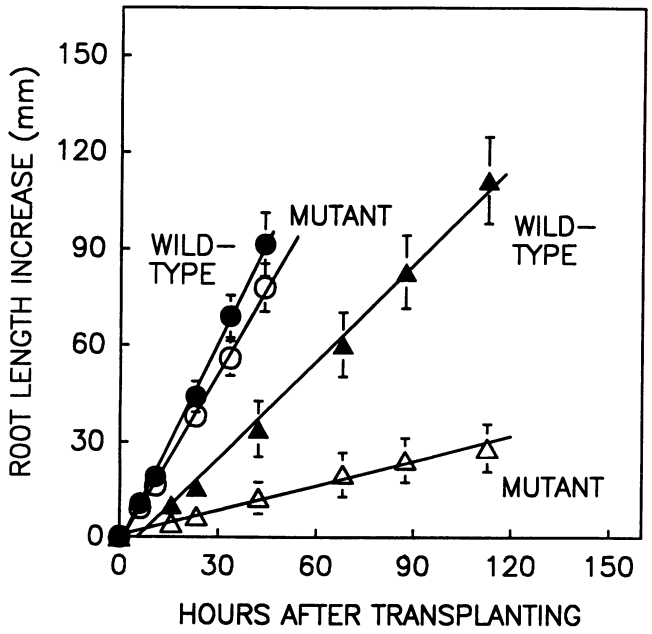

Figure 2. Elongation of the primary root of viviparous mutant (vp 5) and wild-type maize seedlings growing in high (−0.03 MPa, circles) or low (−1.60 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 4 to 22 roots. Experiments were repeated 1 to 4 times with similar results. Error bars are not shown when smaller than the symbols.

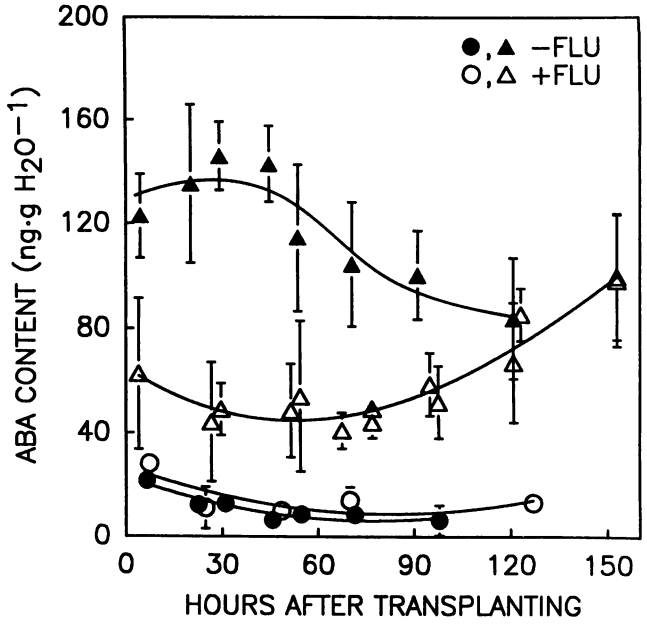

Figure 3. Effect of fluridone (FLU) on ABA content in the apical 10 mm of the primary root of maize (FR27 × FRMo17) seedlings growing in high (−0.03 MPa, circles) or low (−1.60 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 3 to 6 measurements of 3 to 6 root tips each, combined from two experiments. Error bars are not shown when smaller than the symbols.

Taken together, results of the primary root studies showed that in both the fluridone and mutant approaches, reduced accumulation of ABA at low water potential was associated with further inhibition of root growth than that caused by low water potential alone. This interpretation required that neither treatment cause a large effect on growth at high water potential, as was the case, and is strengthened by the concurrent recovery of root elongation rate and ABA content in fluridone-treated seedlings at low water potential to values observed in untreated seedlings (Figs. 1 and 3).

Effect of Fluridone on Shoot Growth at High and Low Water Potential

At low water potential (approximately −0.3 MPa), treatment with fluridone produced an opposite effect on shoot growth to that observed on root growth: treatment with fluridone actually promoted shoot elongation (Fig. 4). In fact, within the first 5 h after transplanting, shoots of fluridone-treated seedlings at low water potential elongated at about the same rate (2.1 mm h^{-1}) as those of untreated seedlings at high water potential (2.2 mm h^{-1}). In contrast, shoots of untreated

Table I. *Content of ABA in the Apical 10 mm of the Primary Root of Viviparous Mutant (vp 5) and Wild-Type Maize Seedlings at Various Times after Transplanting to High or Low Water Potential Vermiculite*

Values are ng g H_2O^{-1} ± SD of 3 to 5 samples of 3 to 6 root tips each.

Treatment	High Water Potential (−0.03 MPa)		Low Water Potential (−1.60 MPa)	
	20 h	45 h	45 h	75 h
Wild type	15 ± 2	13 ± 4	116 ± 13	149 ± 35
Mutant	12 ± 5	12 ± 4	46 ± 10	35 ± 8

seedlings at low water potential elongated at a rate of only 1.2 mm h^{-1} during the same period. At high water potential, treatment with fluridone had little effect on shoot elongation.

Shoot Growth of Mutant and Wild Type at High and Low Water Potential

Shoot growth responses to low water potential of the mutant and wild type were similar, respectively, to those of fluridone-treated and untreated seedlings of the commercial variety (*cf.* Figs. 4 and 5). During the first 6 h after transplanting to −0.3 MPa, shoots of mutant seedlings elongated at approximately twice the rate of the wild type (0.9 mm h^{-1} compared with 0.4 mm h^{-1}; Fig. 5). In contrast, shoot elongation rate of mutant seedlings at high water potential was approximately 14% less than that of the wild type during the same period (1.2 mm h^{-1} compared to 1.4 mm h^{-1}). The reduced elongation rate of mutant shoots at high water potential was explained by the presence of necrotic tissue near the coleoptile tips of most seedlings. This suggests that some precocious shoot elongation may have occurred during kernel development (without visible piercing of the pericarp), which was followed by death of the tissue upon desiccation. Nevertheless, shoots of mutant seedlings were still capable of more rapid elongation than the wild type at low water potential.

Time Course of ABA Content in the Shoot Growing Zone

The time course of ABA content was measured in the upper mesocotyl to document that accumulation of ABA was indeed reduced in the shoot growing regions of fluridone-treated and mutant seedlings at low water potential (Fig. 6; Table II). Low water potential caused a large increase in ABA content in untreated and wild-type seedlings, as expected. ABA levels were reduced considerably in fluridone-treated and mutant seedlings at the same water potential. The treatments had little effect on ABA levels at high water potential.

Taken together, results of the shoot studies showed that in both the fluridone and mutant approaches, reduced accumulation of ABA was associated with less growth inhibition at low water potential. Shoot growth inhibition was completely prevented during the first 5 h of the low water potential treatment in the fluridone-treated seedlings.

DISCUSSION

The results presented here provide strong evidence for a dual role of endogenous ABA in the growth responses of maize seedlings to low water potentials: elevated levels of ABA act differentially to maintain primary root growth and inhibit shoot growth. This conclusion is based on the similar results obtained using either fluridone or the vp 5 mutant to reduce the accumulation of ABA in the root and shoot growing zones at low water potentials. In both cases there was severe inhibition of root elongation and promotion of shoot elongation compared to untreated and wild-type seedlings growing at the same water potentials.

Our results indicate that the effects of reducing endogenous ABA levels at low water potentials were exerted directly on the growth of the individual organs. Certainly, the growth responses were independent of effects of ABA on stomata and overall plant water balance because seedlings were grown in the dark under conditions of near-zero transpiration. Also, effects on root growth were clearly independent of effects on shoot growth because root experiments were conducted at a water potential of approximately −1.6 MPa, which completely inhibited shoot growth (25). On the other hand, the promotion of shoot elongation by fluridone at a water potential of −0.3 MPa was concomitant with inhibition of root elongation (data not shown). Dry weight determinations in similar experiments, however, showed that the promotion of shoot dry weight by fluridone exceeded (by approximately twofold) the reduction of root dry weight. Therefore, the promotion of shoot growth was not simply the indirect result of root growth inhibition.

The value of manipulating endogenous ABA levels by chemical or genetic means, as an alternative to external

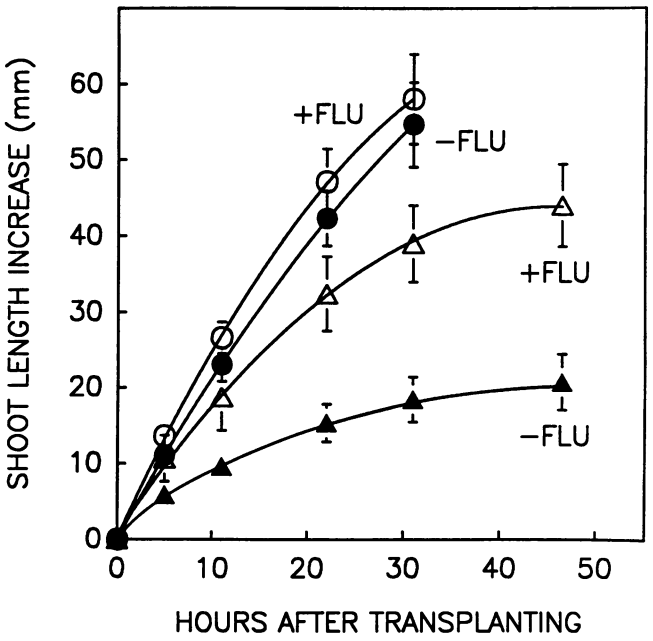

Figure 4. Effect of fluridone (FLU) on elongation of the shoot of maize (FR27 × FRMo17) seedlings growing in high (−0.03 MPa, circles) or low (−0.30 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 34 to 40 shoots. Experiments were repeated once with similar results. Error bars are not shown when smaller than the symbols.

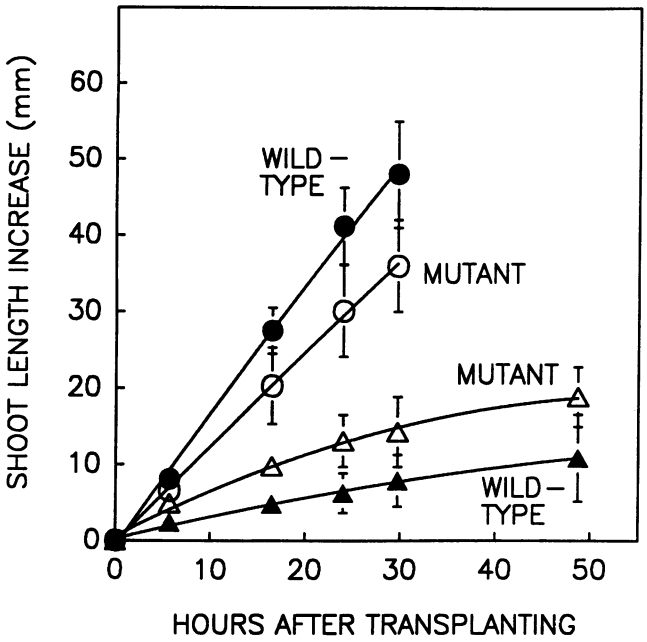

Figure 5. Elongation of the shoot of viviparous mutant (vp 5) and wild-type maize seedlings growing in high (−0.03 MPa, circles) or low (−0.30 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 8 to 13 shoots. Error bars are not shown when smaller than the symbols.

applications, for investigations into its physiological roles has been recognized (13, 16), but this approach has not been taken in previous studies of the role of ABA in growth responses of water-stressed plants. The dependence of maize primary root elongation at low water potential on high levels of ABA, suggested by our results, could not have been inferred from published results of studies at high water potential. Although root growth promotion by applied ABA has been reported in maize (15, 19), high concentrations of applied and endogenous ABA have generally been associated with root growth inhibition (19, 22). The case for using water-stressed tissue in studies of the role of ABA in responses to low water potentials is strengthened by the findings of Hartung *et al.* (8), who showed that ABA partitioning can be altered by water stress-induced changes in apoplastic pH. In addition, it has been suggested that differences in compartmentation exist between endogenous and applied ABA (4, 19).

In contrast to the primary root results, the finding that reduced levels of endogenous ABA were associated with less inhibition of shoot growth at low water potential is complementary to many reports that showed an inhibition of shoot growth in response to ABA application to well-watered plants (1, 5, 11, 28, 29). In soybean, Creelman *et al.* (5) observed that application of ABA to well-watered seedlings resulted in a maximum hypocotyl growth inhibition which was only 60% of that caused by transplanting to low water potential (−0.3 MPa) vermiculite. In our study, however, fluridone treatment completely prevented inhibition of shoot growth during the first 5 h after transplanting to −0.3 MPa. This suggests that the inhibition of shoot growth, at least for that duration, was caused entirely by the rise in endogenous ABA. Accordingly, the supply of water for tissue expansion was not a limiting factor. This differs from the conclusion of Nonami and Boyer (17) that the initial inhibition of soybean hypocotyl growth upon transplanting to vermiculite at −0.3 MPa was caused by collapse of the water potential gradient between the xylem and growing cells, which was followed only after 5 to 10 h from transplanting by metabolic limitations to growth.

In both the inhibitor-treated and mutant seedlings, reduced accumulation of ABA at low water potentials was associated with inhibition of carotenoid synthesis (in both cases the conversion of phytoene to phytofluene is affected [7]). The similarity of growth responses between the two approaches indicates that other potential effects of fluridone on growth were negligible at the concentration used. However, the reduced synthesis of carotenoids raises a possibility that both treatments were associated with general metabolic inhibition, which might explain the severe inhibition of root growth which occurred at low water potential. This seems unlikely for the following reasons. In all studies, seedlings were grown in the dark to circumvent any effects of the presence of chloroplasts and light-inducible carotenoids on growth. Also, growth of roots at high water potential (maximum growth rate) was little affected by either treatment. Moreover, shoot growth of fluridone-treated and mutant seedlings was actually promoted at low water potential. Thus, it is highly unlikely that the two treatments caused a general metabolic inhibition only in roots and only at low water potential.

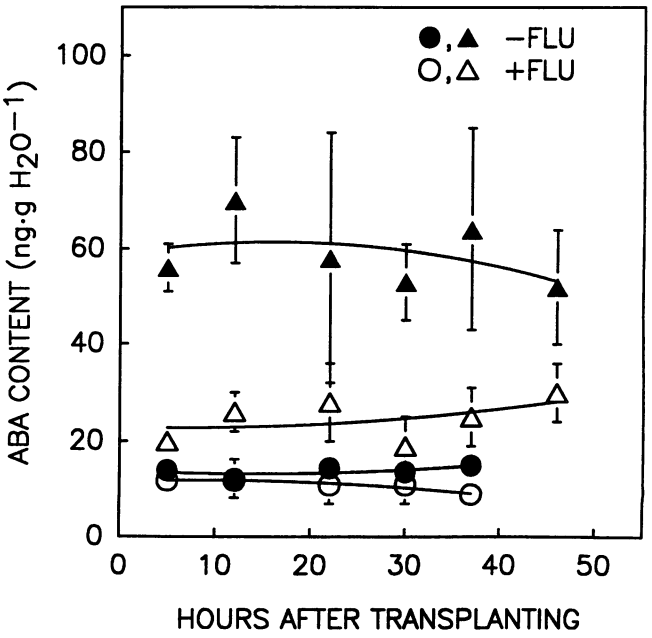

Figure 6. Effect of fluridone (FLU) on ABA content in the uppermost 15 mm of the mesocotyl of maize (FR27 × FRMo17) seedlings growing in high (−0.03 MPa, circles) or low (−0.30 MPa, triangles) water potential vermiculite. Data points represent the mean ± SD of 3 to 7 measurements of 2 to 5 sections each, combined from two experiments. Error bars are not shown when smaller than the symbols.

Table II. *Content of ABA in the Uppermost 15 mm of the Mesocotyl of Viviparous Mutant (vp 5) and Wild-Type Maize Seedlings at Various Times after Transplanting to High or Low Water Potential Vermiculite*

Values are ng g H_2O^{-1} ± SD of 3 to 5 samples of 2 to 5 sections each.

Treatment	High Water Potential (−0.03 MPa)		Low Water Potential (−0.30 MPa)	
	10 h	30 h	24 h	49 h
Wild type	16 ± 4	11 ± 3	44 ± 6	64 ± 8
Mutant	14 ± 1	11 ± 3	17 ± 3	31 ± 4

To confirm that reduced accumulation of ABA was indeed responsible for the severe inhibition of root elongation in fluridone-treated and mutant seedlings at low water potential, it is desirable that the inhibition is reversed when the internal ABA level is raised by exogenous application of the hormone. We attempted this experiment by adding various concentrations of ABA to the water mixed with the vermiculite into which the seedlings were transplanted. A very high external ABA concentration (1 mM) was required to raise the volume-averaged ABA level in the apical 10 mm of fluridone-treated roots growing at −1.6 MPa to that of untreated roots at the same water potential. This treatment resulted in further inhibition of root elongation. The negative result of this experiment must be interpreted with caution, however. As previously mentioned, cellular compartmentation and tissue distribution are probably different for exogenous and endogenous ABA, and this problem is likely to be greater at high applied concentrations. Indeed, preliminary studies have shown that fluridone-induced inhibition of root elongation rate (and ABA accumulation) can be reversed by applying ABA at much lower concentrations when the experiments are conducted at a water potential of −0.3 MPa (26). A detailed analysis of this response will be reported in a subsequent paper.

LITERATURE CITED

1. **Bensen RJ, Boyer JS, Mullet JE** (1988) Water deficit-induced changes in abscisic acid, growth, polysomes, and translatable RNA in soybean hypocotyls. Plant Physiol **88:** 289–294
2. **Boyer JS, Knipling EB** (1965) Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Natl Acad Sci USA **54:** 1044–1051
3. **Bradford KC, Hsiao TC** (1982) Physiological responses to moderate water stress. *In* OL Lange, PS Nobel, CB Osmond, H Zeigler, eds, Encyclopedia of Plant Physiology (New Series), Vol 12B, Physiological Plant Ecology II. Springer-Verlag, Berlin, pp 263–324
4. **Bray EC, Zeevart JAD** (1986) Compartmentation and equilibration of abscisic acid in isolated *Xanthium* cells. Plant Physiol **80:** 105–109
5. **Creelman RA, Mason HS, Bensen RJ, Boyer JS, Mullet JE** (1990) Water deficit and abscisic acid cause differential inhibition of shoot *versus* root growth in soybean seedlings. Analysis of growth, sugar accumulation, and gene expression. Plant Physiol **92:** 205–214
6. **Davies WJ, Metcalfe J, Lodge TA, da Costa AR** (1986) Plant growth substances and the regulation of growth under drought. Aust J Plant Physiol **13:** 105–125
7. **Fong F, Koehler DE, Smith JD** (1983) Fluridone induction of vivipary during maize seed development. *In* JE Kruger, DE LaBerge, eds, III International Symposium on Pre-harvest Sprouting in Cereals. Westview Press, Boulder, CO, pp 188–196
8. **Hartung W, Radin JW, Hendrix DL** (1988) Abscisic acid movement into the apoplastic solution of water-stressed cotton leaves. Role of apoplastic pH. Plant Physiol **86:** 908–913
9. **Hsiao TC, Bradford KJ** (1983) Physiological consequences of cellular water deficits. *In* HM Taylor, WR Jordan, TR Sinclair, eds, Limitations to Efficient Water Use in Crop Production. American Society of Agronomy, Madison, WI, pp 227–265
10. **Jones H, Leigh RA, Tomos AD, Wyn Jones RG** (1987) The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots. Planta **170:** 190–197
11. **Kutschera U, Schopfer P** (1986) Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta **167:** 527–535
12. **Meyer RF, Boyer JS** (1981) Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials. Planta **151:** 482–489
13. **Moore R, Smith JD** (1984) Growth, graviresponsiveness, and abscisic acid content of *Zea mays* seedlings treated with fluridone. Planta **162:** 342–344
14. **Moore R, Smith JD** (1985) Graviresponsiveness and abscisic acid content of roots of carotenoid-deficient mutants of *Zea mays* L. Planta **164:** 126–128
15. **Mulkey TJ, Evans ML, Kuzmanoff KM** (1983) The kinetics of abscisic acid action on root growth and gravitropism. Planta **157:** 150–157
16. **Neill SJ, Horgan R** (1985) Abscisic acid production and water relations in wilty tomato mutants subjected to water deficiency. J Exp Bot **36:** 1222–1231
17. **Nonami H, Boyer JS** (1989) Turgor and growth at low-water potentials. Plant Physiol **89:** 798–804
18. **Pilet P-E, Chanson A** (1981) Effect of abscisic acid on maize root growth. A critical examination. Plant Sci Lett **21:** 99–106
19. **Pilet P-E, Saugy M** (1987) Effect on root growth of endogenous and applied IAA and ABA. A critical reexamination. Plant Physiol **83:** 33–38
20. **Quarrie SA, Whitford PN, Appleford NEJ, Wang TL, Cook SK, Henson IE, Loveys BR** (1988) A monoclonal antibody to (S)-abscisic acid: its characterization and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves. Planta **173:** 330–339
21. **Rosher PH, Jones HG, Hedden P** (1985) Validation of a radioimmunoassay for (+)-abscisic acid in extracts of apple and sweet pepper tissue using high-pressure liquid chromatography and combined gas chromatography-mass spectrometry. Planta **165:** 91–99
22. **Saugy M, Mayor G, Pilet P-E** (1989) Endogenous ABA in growing maize roots: light effects. Plant Physiol **89:** 622–627
23. **Sharp RE, Davies WJ** (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta **147:** 43–49
24. **Sharp RE, Davies WJ** (1989) Regulation of growth and development of plants growing with a restricted supply of water. *In* HG Jones, TL Flowers, MB Jones, eds, Plants Under Stress. Cambridge University Press, London, pp 72–93
25. **Sharp RE, Silk WK, Hsiao TC** (1988) Growth of the maize primary root at low water potentials. I. Spatial distribution of expansive growth. Plant Physiol **87:** 50–57

26. **Sharp RE, Voetberg GS, Saab IN** (1990) Confirmation that ABA accumulation is required for maize primary root growth at low water potentials. Plant Physiol (abstract No. 35) **93:** S-7
27. **Stewart CR, Voetberg GS, Rayapati PJ** (1986) The effects of benzyladenine, cyclohexamide, and cordycepin on wilting-induced abscisic acid and proline accumulation in barley leaves. Plant Physiol **82:** 703–707
28. **Van Volkenburgh E, Davies WJ** (1983) Inhibition of light-stimulated leaf expansion by abscisic acid. J Exp Bot **34:** 835–845
29. **Watts S, Rodriguez JL, Evans S, Davies WJ** (1981) Root and shoot growth of plants treated with abscisic acid. Ann Bot **47:** 595–602
30. **Westgate ME, Boyer JS** (1985) Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. Planta **164:** 540–549
31. **Zeevart JAD, Creelman RA** (1988) Metabolism and physiology of abscisic acid. Annu Rev Plant Physiol and Plant Mol Biol **39:** 439–473